Gorgosterol
Cholesterol
Bacterial degradations
Model experiments

Gorgostérol
Cholestérol
Dégradations bactériennes
Expériences modèles

Hypermethylated side chain marine sterols: aspects of the gorgosterol-cholesterol bio-ecological relationships from model experiments

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ABSTRACT

Gorgosterol, a C_{30}-sterol with a hypermethylated side chain, is biosynthezised by unicellular algae (zooxanthellae) and is found in some marine invertebrates. The coexistence of gorgosterol 1 with cholesterol 2 in marine invertebrates has been considered previously from the adaptative view point. A 1 : 1 mixture of gorgosterol and cholesterol was allowed to ferment in sea water in a model experiment using a pool of marine bacteria. After this experiment, the sterols were recovered in a 1 : 9 ratio, showing the greater sensitivity of the gorgosterol side chain towards bacterial degradation. The bio-ecological consequence of this observation is discussed and the general situation of dissolved or particulate branched chain sterols in the oceans is reconsidered.

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RÉSUMÉ

Stérols marins à chaîne latérale hyperméthylée : aspects des relations bio-écologiques gorgostérol-cholestérol à la suite d'expériences faites sur des modèles

Le gorgostérol 1 est un stérol marin en C_{30}, à chaîne latérale hyperméthylée, trouvé chez divers Invertébrés marins mais biosynthétisé par des algues unicellulaires (zooxanthelles). La co-existence du gorgostérol 1 avec le cholestérol 2 chez des Invertébrés marins a précédemment été envisagée du point de vue de l'évolution. Nous avons effectué des expériences modèles en utilisant un mélange 1 : 1 de gorgostérol et de cholestérol, dans des fermentations en eau de mer ensemencée par des bactéries marines. A la suite de ces essais, les stérols récupérés sont respectivement dans la proportion 1 : 9 mettant en évidence la plus grande sensibilité de la chaîne latérale du gorgostérol envers les dégradations bactériennes. Les conséquences de ces observations en milieu marin, concernant la situation bio-écologique de ces deux stérols, sont discutées en fonction de la recherche d'une « raison d'être » aux hyperméthylations. A partir d'un ensemble de résultats, la situation générale des stérols à chaîne ramifiée, dissous ou particulaires, rencontrés dans les océans, est également discutée.

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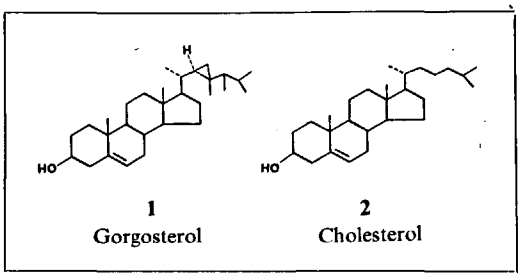
INTRODUCTION

Gorgosterol is the first of several naturally occurring marine sterols containing a side-chain cyclopropyl group (Hale *et al.*, 1970) which has been isolated from marine invertebrates such as the Cnidarians and the Gorgonians. And numerous sterols possessing hypermethylated side chains of the same sort have been found in the marine environment (for reviews see for example Ling *et al.*, 1970; Schmitz, 1978). The biosynthesis of gorgosterol by unicellular algae in culture (the dinoflagellate *Peridinium foliaceum*) has been demonstrated (Withers *et al.*, 1979) thus indicating the possible role of algal symbionts in such hypermethylations.

Several studies of dissolved and particulate sterols in sea water (Saliot, Barbier, 1973; Barbier *et al.*, 1981; Gagosian, 1975; 1976) have not reported the presence of

gorgosterol. Among the possible reasons for this absence would be the very low levels (below detection limits) but also the sensitivity of this sterol toward biological degradation in the marine environment. Is the hypermethylation of the side chain in gorgosterol and in sterols in general, a protective mechanism towards oxidation and bacterial degradation? This question is of some importance considering the existence of the C_{26}, C_{27}, C_{28}, C_{29} sterols as constituent of the oceanic organic matter.



1 Gorgosterol 2 Cholesterol

The bio-ecological significance of such methylations is unknown and an hypothesis (Boutry *et al.*, 1979) was proposed following which branched side chains would oppose to bacterial degradation. Many marine invertebrates are the support of colonies of unicellular algae such as zooxanthellae and of bacteria. As most marine invertebrates do not biosynthezise sterols which are however necessary components of cell membranes, they have to find them in their food or get them from their symbionts. In opposition, bacteria do not synthezise sterols but perform the oxidative degradation. Competitions between colonies of zooxanthellae and bacteria are making of the whole system a very complex one.

Having studied the action of a pool of marine bacteria collected from filter-feeding marine invertebrates (mussels) on the degradation of sterol side chains (Boutry, Barbier, 1981 *a* and *b*) we also decided to observe the relative sensitivity in these conditions of an hypermethylated sterol, namely gorgosterol 1, compared to cholesterol 2. The results from these experiments are now presented and discussed.

MATERIAL AND METHODS

The detailed conditions of the bacterial cultures have been described previously (Boutry, Barbier, 1981 *b*). The experiment was performed in 1 l enriched artificial sea water, with 1 mg gorgosterol, 1 mg cholesterol (in acetone-tween) and the sea water contained in 12 mussels *Mytilus edulis*. The cultures were kept in the dark to avoid photosynthesis by unicellular algae, at 20°C for about 10 days. The broth was directly saponified for 1 hour at 90°C after adding 80 g NaOH. The unsaponifiable fraction was then extracted twice by ether following the usual technique.

The free-OH sterol fraction was isolated by preparative thin layer chromatography (TLC) on SiO_2, developing with hexane-ethyl acetate 7 : 3 (visualisation by H_2SO_4 50%, 110°C) and marking by lateral deposits of authentic cholesterol for the determination of the R_f (0.40). The collected TLC SiO_2 powder was extracted by ether and the products analysed by mass spectrometry using an AEI MS9 spectrometer and by gas liquid chromatography as previously described. The sterol determinations were repeated on acetates prepared by short action of two drops of acetyl chloride at 60°C and drying the reaction mixture *in vacuo* before analysis.

RESULTS AND DISCUSSION

Gorgosterol 1 and cholesterol 2 have been introduced in a fermentation using a pool of marine bacteria, in the ratio 1 : 1. In the reported conditions, the relative proportions of recovered 1 and 2 are (from gas chromatography) 1 : 9 respectively. Investigation of the sea water contained in the same number of mussels as used in the experiment showed the contribution of these sterols to be negligible. A blank experiment dissolving gorgosterol and cholesterol (1 : 1) and extracting in the same conditions after 10 days without fermentation, furnished a 90% recovery of the products after saponification and TLC, still indicating a 1 : 1 ratio. However, the control showed that mass spectrometry was giving lower values than expected for gorgosterol (determinations at 180 and 220°) probably due to the feable volatility comparatively with cholesterol. Thus, values obtained from gas chromatography have been considered for the determination rather than those from mass spectrometry (ions at *m/e* 408 for gorgosteryl acetate and 368 for cholesteryl acetate, in agreement with the loss of acetic acid in the apparatus $(M\text{-}60)^+$).

As bacteria do not biosynthezise sterols and because non-photosynthetic conditions have been used, the ratio of the two sterols recovered after fermentation can be interpreted in terms of relative sensitivity towards bacterial degradation.

In terms of evolution, the hypermethylation of the sterol side chain leading to gorgosterol may lead to preservation of the sterol structure in a symbiotic zooxanthellae-invertebrate relationship but apparently not in a zooxanthellae-bacteria interaction. When present dissolved in sea water, such substances may be readily attacked and degraded by planktonic microorganisms.

Anyhow, the reason of why such hypermethylation mechanisms exist is still a mystery and even if an anarchic intervention of the methylasic system of the host dinoflagellates can not be excluded, the occurrence of gorgosterol should be considered in terms of evolution. The marine environment is rich in sterols which are not found in continental fauna and flora and there is still a need for model experiments to understand such differences.

The concentration of sterols in sea water over time, depends on their relative sensitivities towards physical agents such as oxygen and on planktonic microorganisms. Some results (Boutry, Barbier, 1981 *a* and *b*) indicate that the side chain of branched chain sterols are easily attacked by marine bacteria. Turnover times for sterols in the Sargasso Sea have been calculated (Gagosian, Nigrelli, 1979) and they give an idea of the relative resistance (versus sensitivity) of the different sterols present in the oceans. The comparative photosensitivity of cholesterol, 24-methylene cholesterol, fucosterol and desmosterol has shown desmosterol and

24-methylene cholesterol to be highly sensitive to light and cholesterol to be relatively more resistant (Barbier, 1980, unpublished results). These results demonstrate that sterols obtained from the oceans by extraction procedures (see for examples Saliot, Barbier, 1973; Gagosian, 1975; 1976) are indeed a statistic representation of a complex system.

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Journées du GABIM

Brest 18, 19 et 20 novembre 1981

Centre Océanologique de Bretagne

Indices biochimiques et milieu marin

Biochemical indices and marine processes

Les 18, 19 et 20 novembre 1981, le Groupement pour l'Avancement de la Biochimie Marine (GABIM) organisera sa réunion annuelle à Brest sur le thème « Indices biochimiques et milieu marin ».

Ce thème a pour objet de faire le point sur les apports actuels, les perspectives et les limites de la biochimie pour les études écologiques et physiologiques en milieu marin.

De nombreux indices biochimiques sont apparus récemment dans le domaine de l'écologie (substances dissoutes, milieu particulaire, maillons primaire, secondaire et tertiaire), la physiologie (contrôle d'élevages aquacoles, tests sublétaux et perturbations physiologiques), la dynamique des populations (traceurs génétiques). Il s'agira de faire un bilan critique de ces approches.

Les inscriptions doivent être prises auprès du Secrétariat du GABIM.

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As in the last years, the French Group for the Advancement of Marine Biochemistry (GABIM) is preparing its annual meeting. This year, the meeting will be held in Brest (November 18-19-20). The selected topics will deal with biochemical indices in marine processes.

Biochemistry is an approach recently developped in the field of marine ecological studies. It already covers topics such as dissolved organic matter, particulate matter, primary, secondary, tertiary production. Marine biochemistry is also important in physiological studies applied to topics such as aquaculture, pollutants and other physiological perturbations, or population dynamics and genetics (tracers). The biochemical approaches in these fields will be reviewed and discussed.

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